Lactose intolerance

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Results and discussion

1. Fermentation of lactose by the colonic microbiota may play a role in lactose intolerance

The lactose tolerant and intolerant subjects did not differ in LDI or OCTT of lactose, which suggests the involvement of other pathologic mechanisms in lactose intolerance. We hypothesize that colonic metabolism of lactose is one of these mechanisms **(Appendix 1)**. During the *in vitro* incubation of feces with lactose, the lactose intolerant group produced D- and L-lactate, acetate, propionate and butyrate significantly faster than the tolerant group. In the intolerant group, the amount of acetate, propionate, butyrate and L- lactate produced was higher than that in the tolerant group. The results indicate that a faster and higher production of microbial intermediate and end metabolites during colonic fermentation of lactose, may be related to the development of lactose-induced symptoms **(Appendix 3)**. However, the degree and rate of lactose hydrolysis in the colon does not play a role. During colonic fermentation, lactose is first hydrolyzed to glucose and galactose, which is catalyzed by ß-galactosidase. We found that bacterial ß-galactosidase activity is abundant in the colon as 80.6% (mean, SD: 12.1, range: 47.8%-100%) of the cultured fecal bacteria possess this activity. The lactose tolerant and intolerant subjects did not differ in the percentage or composition of the bacteria with ß-galactosidase activity or ß-galactosidase activity in feces **(Appendix 2)**. We assume that lactose itself will not present a large osmotic burden to the colon as it should be quickly degraded by the colonic microbiota. This assumption is supported by observations that the tolerant and intolerant groups did not differ in the rate or degree of hydrolysis of lactose or production of glucose and galactose **(Appendix 3)**. We propose that after lactose is hydrolyzed, the subsequent fermentation of glucose and galactose may play a role in the pathophysiology of lactose intolerance.

Whether colonic fermentation of lactose would influence lactose intolerance, either aggravates or alleviates it, depends on the balance between the ability of the colonic microbiota to ferment lactose and the ability of the colon to remove the fermentation metabolites. We assume that the absorption rate of the colon is not sufficient to remove all the SCFA and other metabolites produced from rapid

fermentation of lactose. This leads to temporary accumulation of SCFA and other metabolites in the colon, which plays a role in the onset of lactose-induced symptoms possibly through their osmotic load, altering intestinal motility or causing colonic hypersensitivity.

The *in vitro* results suggest that colonic fermentation of lactose may play a role in lactose intolerance. This provides the basis for the following studies: (i) to investigate whether the colonic microbiota could be modulated by dietary supplementation for the purpose of alleviating symptoms; (ii) to explore proteomics techniques to study metabolic pathways of lactose fermentation by the colonic microbiota; (iii) to design an *in vivo* study to verify the observations of the *in vitro* study.

2. Yogurt and bifidobacteria supplementation modifies the colonic microbiota and alleviates lactose-induced symptoms in lactose intolerant subjects

Our results suggest that colonic fermentation of lactose may play a role in lactose intolerance **(Appendix 3)**. This raises the question whether we can modulate the composition and metabolic activities of the colonic microbiota in such a way that lactose intolerance could be attenuated. In this study **(Appendix 4)**, 2-w supplementation of probiotic bacteria *Bifidobacterium longum* and a yogurt enriched with *Bifidobacterium animalis* increased the numbers of total cells, total bacteria and *Eubacterium rectale/Clostridium coccoides* group and ß-galactosidase activity in feces of lactose intolerant subjects. The supplementation did not increase the endogenous lactase activity in the small intestine. Symptoms of lactose intolerance decreased after the supplementation. The increase in bacterial numbers could be attributed to the lactose contained in the yogurt which can be considered as a prebiotic for people with lactose maldigestion (162,163). Reduction in symptoms could be caused by adaptation to lactose consumption (80) and changes in the composition and metabolism of the colonic microbiota. In conclusion, supplementation of yogurt and a probiotic strain modified the amount and probably the metabolic activities of the colonic microbiota of lactose intolerance subjects.

The changes in the colonic microbiota may play a role in alleviation of intolerant symptoms.

3. Exploring proteomic techniques for studying lactose metabolism by the colonic microbiota

In the above studies **(Appendix 2-4)**, the composition of the colonic microbiota was determined by FISH, DGGE and an X-gal assay, and the metabolic activities of the colonic microbiota were investigated by *in vitro* incubation and by determination of enzyme activities. Development of new techniques will facilitate the studies on the role of the colonic microbiota in health and disease. Proteomic techniques might aid to interpret the metabolic pathways of lactose metabolism by the colonic microbiota. In this study **(Appendix 5)**, the SELDI-TOF MS Proteinchip technology and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) could discern differences in protein expression of bifidobacteria grown on lactose, glucose and galactose. With LC-MS/MS, proteins related to *Bifidobacterium* were identified, which included enzymes for metabolism of lactose, glucose and galactose. The applied approaches are promising in studying metabolism of lactose and other substrates in a complex bacterial ecosystem such as the colonic microbiota, but need further development. For instance, when fractionation by SDS-PAGE and identification by LC-MS/MS is combined with stable isotope labeling, it will facilitate identification of proteins of which expression is induced by specific substrates.

4. Lactose accelerates the oro-cecal transit in lactose maldigesters

Studies on digestion and transit of lactose in the small intestine would help to clarify possible involvement of the colon in lactose intolerance. In the study **(Appendix 1),** we observed that ompared to glucose, lactose triggers a faster transit in the small intestine in lactose maldigesters, but not in digesters. The accelerated transit is not the result of intestinal distention caused by osmotic load from malabsorbed lactose as suggested earlier (31,97). Based on LDI, we estimated the

amount of fluid the maldigested lactose would attract to the intestine in lactose digesters and maldigesters. The amounts are unlikely to cause intestinal distention and the difference in the amount between digesters and maldigesters is unlikely to cause the difference in OCTT. We hypothesize that presence of maldigested lactose in the intestinal lumen plays a role in the alteration of intestinal transit by affecting the intrinsic factors that regulate intestinal motility. Postprandial motility of the gastrointestinal tract is controlled by nerves, hormones and paracrine mediators (164). It might be possible that undigested lactose alters the motility of the intestine by stimulating the secretion of certain gastrointestinal hormones, or by stimulating the neural activities of certain chemosensitive receptors or osmoreceptors.

We speculate that this hypothesis can be extended to explain the accelerated intestinal transit in malabsorption of other sugars and some food components, for instance, fructose and sorbitol (165-170). Malabsorption of lactose, fructose and sorbitol can be related, as fructose and sorbitol malabsorption are common when lactose malabsorption is present (168).

The lactose tolerant and intolerant subjects did not differ in degree of lactose digestion (LDI) or OCTT of lactose. This suggests the involvement of other pathologic mechanisms in lactose intolerance, *e.g.* the colonic metabolism of lactose.

Future perspectives

Comparison of colonic metabolism of lactose *in vivo* between lactose tolerant and intolerant subjects using stable isotopes

Our *in vitro* study indicates that a faster and higher production of microbial intermediate and end metabolites during colonic fermentation of lactose, may play a role in lactose-induced symptoms **(Appendix 3)**. However, the *in vitro* system may not be a perfect reflection of the *in vivo* situation. The culturing conditions are different from those in the colon. Colonic factors, *e.g.* removal of the metabolites, gut secretions and immunology and interaction with mucosal cells, are not studied. Therefore, our *in vitro* observations need to be verified by *in vivo* studies.

In vivo studies on colonic metabolism of certain substrates with humans are scant as they are hampered by difficulties in sampling *in situ* and quantitative delivery of substrates to the colon. To circumvent these difficulties, several approaches using stable isotopes can be considered.

1. Oral administration of ^{13}C-lactose-ureide

Background: The human gut tissue possesses no allantoicase-like activity to split the bond between glycosyl and ureide (171). Therefore, glycosyl ureides cannot be absorbed in the small intestine. In the colon, glycosyl ureides can be degraded by *Clostridium innocuum* strains which belong to the normal intestinal microbiota of infants and adults (172). After the glycosyl-ureide bond is split, the glycosyl and ureide will be further metabolized by colonic bacteria. Glycosyl ureides are used as a marker for measurement of the OCTT (173).

Approaches: Tracer amount of ^{13}C-lactose-ureide and 25 g of lactose dissolved in water will be administered orally in lactose maldigesters. Peripheral blood samples will be collected for measurement of ^{13}C-acetate. Breath samples will be collected for determination of OCTT. ^{13}C-lactose-ureide is not absorbed in the small intestine and will enter the colon. After the lactose ureide bond is split, ^{13}C-lactose

will be fermented by the colonic bacteria together with the maldigested unlabeled lactose. The kinetics of ^{13}C-acetate in the peripheral blood will reflect the kinetics of colonic fermentation of maldigested lactose.

Disadvantages: The hydrolysis of the lactose-ureide bond by bacterial enzymes is the rate-limiting step in bacterial degradation of lactose-ureide (174). However, there is no detailed information available on how long this step takes or whether the time varies among individuals. If there is a large inter-individual variation, the fermentation of ^{13}C-lactose-ureide cannot represent the fermentation of lactose.

2. Isotope-dilution technique

Background: The principle of the isotope-dilution technique is as following: in a closed volume at steady-state (production and elimination rates are at equilibrium), the labeled tracer is infused at a constant and known rate until a new steady-state is reached. Blood samples are collected at regular intervals. From the isotopic enrichment in blood at steady-state the production or elimination rates of the metabolite of interest can be calculated (139). The isotope-dilution technique has been used to quantitatively estimate colonic fermentation of non-digestible carbohydrates (137-139,175).

Approaches: Lactose maldigesters will receive a primed, constant and intravenous infusion of [l-^{13}C]acetate for 7 h. 25 g of lactose will be ingested 1 h after the tracer infusion starts. Arterialized venous blood samples will be collected for determination of the total production and the production rate of [l-^{13}C] acetate.

Disadvantages: As absorption of lactose in the small intestine varies among lactose maldigesters, the amount of lactose entering the colon varies. The amount of lactose in the colon may influence the rate of bacterial fermentation. Therefore, the possible differences in total production and the production rate of [l-^{13}C] acetate measured in blood can be derived from the difference in amount of lactose entering the colon.

3. Colon-delivery capsules

Various colon-specific drug delivery systems have been developed (176). The release triggering mechanisms of these capsules can be pH- or time-dependent, microbiota-activated, pressure-dependent, *etc*. However, the delivery capacity of these systems may not be sufficient to deliver the amount of lactose averagely maldigested after ingestion of 25 g of lactose in maldigester (~20 g).

4. Colon-infusion catheters

Different types of catheters have been implanted into the colon for various purposes (104,177). Although the procedure can be experience-dependent and somewhat invasive, colon-infusion catheters allow quantitative delivery of large amounts of substrates. Therefore, for our purpose of studying colonic fermentation of lactose *in vivo*, colon-infusion catheters can be an appropriate approach. An *in vivo* study on colonic fermentation of lactose using stable isotopes delivered via a colon-infusion catheter is proposed (Appendix 6).

Concluding remarks

The results presented in this dissertation suggest that colonic metabolism of lactose, in addition to the small-intestinal lactase activity and transit, may play a role in the pathophysiology of lactose intolerance. During fermentation of lactose by the colonic mictobiota, the rate and magnitude of production of metabolites could be among the factors contributing to the onset of lactose-induced symptoms. This observation implies that in studies on colonic fermentation of carbohydrates, *i.e*. prebiotics, the rate and magnitude of fermentation can be of health relevance.

Dietary supplementation of pre-, pro- or synbiotics provides a promising approach for dietary management of lactose intolerance.